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Thermal Inactivation of Haemagglutinating Activity of Normal and Genetically-Improved Common Bean Varieties: A Kinetic Approach

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ABSTRACT

Two cultivars of Phaseolus vulgaris, *FM-RMC, showing resistance to some plant pathogens and its progenitor, FM-C, without such resistance, were used to study the thermal inactivation of seed haemagglutinating activity (HA). FM-RMC showed a higher level of HA as compared to the FM-C. However, conventional cooking conditions practically abolished HA of both materials. Thermal inactivation of raw extracts of lectins showed biphasic patterns. A developed kinetic model fitted the experimental observations and showed that the HA loss rate of the improved cultivar differed only in a temperature constant.*

INTRODUCTION

Seeds of common bean contain heat-labile proteins that greatly reduce the nutritional value of uncooked beans. Raw beans are toxic when fed to animals and the principal toxicant is lectin or phytohaemagglutinin protein

(Jaffé, 1979; Pusztai *et al.*, 1979). The antinutritional effect of lectins can be partially or wholly eliminated by moist heat treatment applied during cooking (Grant *et al.*, 1982; Thompson *et al.*, 1983; Kadam *et al.*, 1987). Lectins are carbohydrate-binding proteins with haemagglutinating and mitogenic properties that occur in many plant species, especially *Leguminosae* (Liener, 1976; Felsted *et al.*, 1981).

Few studies are available on the thermal inactivation of haemagglutinating activity (HA). These investigations have been performed on the lectins *in situ* (Grant *et al.*, 1982; Thompson *et al.*, 1983; Kadam & Smithard, 1987). In recent reports (Boufassa *et al.*, 1986; Kadam & Smithard, 1987), the lectins were isolated from the seed and the loss of HA in the course of thermal processing followed. These workers found in common that lectins in seeds, or raw extracts from them, exhibited in biphasic pattern of inactivation above certain temperature levels.

To our knowledge no investigations have been carried out on the influence of plant breeding on the haemagglutinating properties of the progeny. This work was undertaken to compare the thermal stability of seed lectins, as assessed by HA, in a normal bean variety in relation to a derived and genetically-improved cultivar.

MATERIALS AND METHODS

Bean samples

The cultivars used for this study (*Phaseolus vulgaris* L.) were sown in plots at the experimental farm during the spring of 1986. The normal variety, flor de mayo (FM-C), without resistance to the common mosaic virus and to bean rust (*Uromyces phaseoli*), was genetically improved by backross breeding into the new derived cultivar, flor de mayo (FM-RMC), with resistance to the cited pathogens. Both old and new materials have a high demand in the Mexican market because of their desirable sensory attributes. Mature seeds were harvested, cleaned and stored in tightly sealed containers at 4°C until used.

Preparation of soaked and cooked samples

Samples for determination of HA were soaked for 16 h in distilled water and drained. Another portion was soaked as described and cooked in fresh water for 86 min at 95°C. Cooking time was determined using the Mattson bean cooker (Juárez *et al.*, 1988). Beans were also soaked and cooked as before but

in soaking water. A final lot was cooked without pre-soaking. All samples were freeze-dried and milled to pass a 100-US mesh sieve.

Lectins extraction

The PBS (phosphate buffer saline)-soluble protein was extracted from the seed flour (1:25, w/v), pH 7·4, in an Ultra-turrax homogeniser at maximum speed for 5 min at room temperature (Felsted *et al.*, 1981). The homogenate was stirred and centrifuged as described by Felsted *et al.* (1975, 1981). Protein content was estimated by microKjeldahl (N × 6·25).

Thermal treatment of extracted lectins from raw beans

The PBS extracts of raw beans from both cultivars were used for this study. The protein content of supernatants was standardised with the same buffer to 5 mg/ml. Series of test tubes (10 × 100 mm) containing 1 ml of this extract were incubated in a water bath at 65, 70, 75, 80 and 85°C for 5, 10, 15, 20, 25, 30, 40, 50 and 60 min. After each treatment, the tubes were immediately frozen in liquid nitrogen, then stored at −60°C until tested. After thawing, each aliquot was assayed for residual HA.

Haemagglutination assays

Starting with 50 μl of the extracts, serial twofold dilutions were made with PBS. Trypsinised rabbit red blood cell suspension (50 μl), prepared according to Lis & Sharon (1972), was then added. HA was expressed as the reciprocal of the highest dilution giving positive agglutination, unless otherwise stated (Thompson *et al.*, 1983).

Kinetic modeling

A kinetic model was developed based on the HA patterns exhibited by the extracted lectins. It was assumed that during the thermal inactivation integral molecules of lectins with full HA give rise to haemagglutinating active subunits and that both, integral molecules and subunits, are inactivated at a rate proportional to their concentration; the inactivation is also a function of temperature. This assumption is based on the fact that haemagglutinin is a family of tetrameric glycoproteins made up of the different combinations of the two subunits termed *E* and *L*. These tetramers differ in their biological properties: *E*4 is the most potent erythroagglutinin and *L*4 is the most potent lymphocyte mitogen, while the three intermediate

forms possess both properties (Leavitt *et al.*, 1977). The system proposed is formed by the following derivatives in relation to time:

$$\dot{L}_1(t) = -\alpha_1 L_1(t)$$
$$\dot{L}_2(t) = -\alpha_2 L_2(t) + \beta L_1(t) \quad (1)$$

where:

$\dot{L}_1(t)$, $\dot{L}_2(t)$ = derivatives in relation to time.
t = heating time.
$L_1(t)$ = amount of HA from integral molecules of lectins in time t.
$L_2(t)$ = amount of HA from active subunits generated from the integral molecules in time t.
α_1 = lectin inactivation ratio as a function of temperature.
α_2 = inactivation ratio of active subunits as a function of temperature.
β = generation ratio of active subunits.

RESULTS AND DISCUSSION

The effect of cooking on the HA of FM-C and FM-RMC cultivars is described in Table 1. Soaking decreased the HA of the raw beans. However, under the cooking conditions used, presoaked and unsoaked samples showed very slight differences in the HA. Interestingly, the improved cultivar showed a higher level of HA as compared to that of the normal variety, but during cooking this activity was also significantly reduced or even abolished. It means that with the conventional cooking procedures

TABLE 1
Influence of Cooking Conditions on the Bean Haemagglutinating Activity[a]

Sample	*Haemagglutinating activity of cultivars*	
	FM-C	*FM-RMC*
Raw	$1{\cdot}15 \times 10^6$	$2{\cdot}32 \times 10^6$
Soaked	$0{\cdot}55 \times 10^6$	$0{\cdot}54 \times 10^6$
Soaked and cooked with fresh water	467	0
Soaked and cooked with soaking water	537	253
Cooked without pre-soaking	0	0

[a] Mean of two determinations. Results in haemagglutinin units/g dry flour.

followed by the consumers (soaking overnight and cooking in boiling water for about 2 h), the antinutritional effect of this factor will be practically eliminated in the new variety.

Changes in HA of the extracted lectins heated at various temperatures and times are shown in Fig. 1 for the FM-C cultivar. Although HA did not decline in any marked way at 65 and 70°C, this activity was clearly sensitive to heat treatment at higher temperatures. Thus when samples were heated at 75, 80 and 85°C the activity was clearly reduced, especially within the first 15 min, although not completely eliminated. The biphasic inactivation pattern obtained at the last three temperatures was in good agreement with the two concomitant first-order reactions with different rate constants observed by Boufassa *et al.* (1986). These workers analysed inactivation of lectins isolated by affinity chromatography heated from 74 to 86°C. Recent studies (Kadam & Smithard, 1987) demonstrated that a biphasic reaction was also exhibited during heat-inactivation of extracted lectins. The effect of heating whole winged beans (Kadam & Smithard, 1987) and common beans (Grant *et al.*, 1982; Thompson *et al.*, 1983) on their HA showed a biphasic graph as well. Interestingly, the biphasic pattern was only observed in these studies at relatively high temperatures (e.g. at or above 80°C, use of autoclaving) by both unextracted and extracted seed lectins, which agrees with the present investigation.

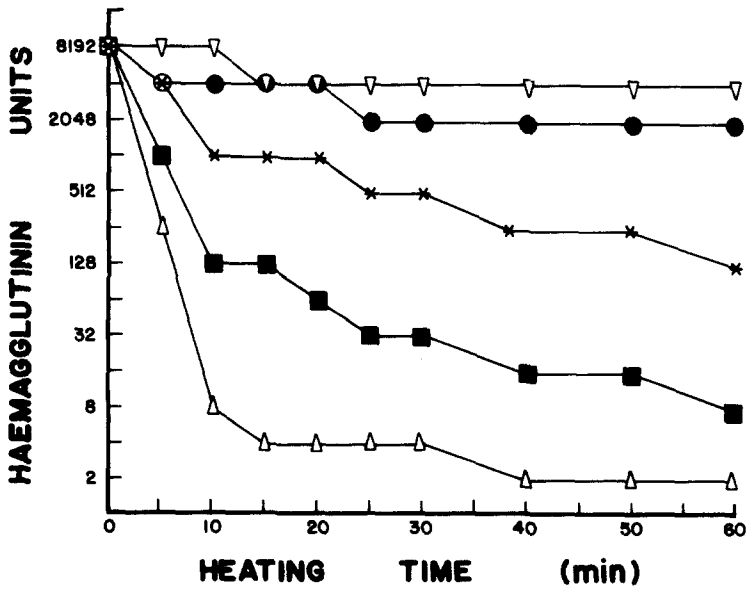


Fig. 1. Inactivation patterns of haemagglutinating activity by raw extracts of lectins from the normal bean cultivar (FM-C).
▽, 65°C; ●, 70°C; ×, 75°C; ■, 80°C; △, 85°C.

Figure 2 shows that the genetically-improved cultivar presented, in general, the same inactivation profiles as the normal variety. However, it should be noted that, as previously indicated, the agglutination capacity of the derived cultivar was higher than that of the FM-C samples.

The solution to the system (1) takes the form of eqns (2(a)) and (2(b)):

$$L_1(t) = L_1(0)\,e^{-\alpha_1 t} \tag{2(a)}$$

$$L_2(t) = L_2(0)\,e^{-\alpha_2 t} + \frac{L_1(0)\beta(e^{-\alpha_2 t} - e^{-\alpha_1 t})}{\alpha_1 - \alpha_2} \tag{2(b)}$$

To calculate α_1, α_2 and β with the experimental results of Fig. 1, the following considerations were taken: $L\,(0) = 8192$ and $L\,(0) = 0$; the HA declined in an exponential way in the first 15 min and the exponent must be very close to α_1; the slope in the slow reaction was close to α_2. The β values were calculated from the relationship between the $L_1(t)$ and $L_2(t)$ profiles. Thus eqn (2(b)) became:

$$L_2(t) = \frac{8192\,\beta(e^{-\alpha_2 t} - e^{-\alpha_1 t})}{\alpha_1 - \alpha_2} \tag{3}$$

The procedure was similar with the experimental data of Fig. 2. Equation (2(b)) became:

$$L_2(t) = \frac{16\,384\,\beta(e^{-\alpha_2 t} - e^{-\alpha_1 t})}{\alpha_1 - \alpha_2} \tag{4}$$

The estimated α_1, α_2 and β parameters of eqn (3) and (4), for the lectins from both cultivars, are given in Table 2. For the two cultivars, the lectin inactivation ratio of integral molecules (α_1) and that of lectin subunits (α_2) increased with temperature, whereas the generation ratio of active subunits (β) declined. These results were in agreement with the formulated assumptions needed to develop the model. All predicted values with this model very closely followed the observed measurements, suggesting that the proposed hypothesis is valid for the thermal inactivation of HA of the *Phaseolus vulgaris* cultivars used in this work.

In conclusion, the disease-resistant cultivar, FM-RMC, showed a higher level of HA than the normal variety, FM-C. This performance agrees with the postulation of the protective role of lectins against plant pathogens (Goldstein, 1981). The conventional household cooking method destroyed the HA of the progenitor and its derived cultivar. The thermal inactivation of extracted lectins from both varieties tended to show biphasic patterns. Also, according to the proposed kinetic model, the HA loss rate of both cultivars was somewhat similar with a difference only in temperature constant. In summary, according to the previous experimental evidence the

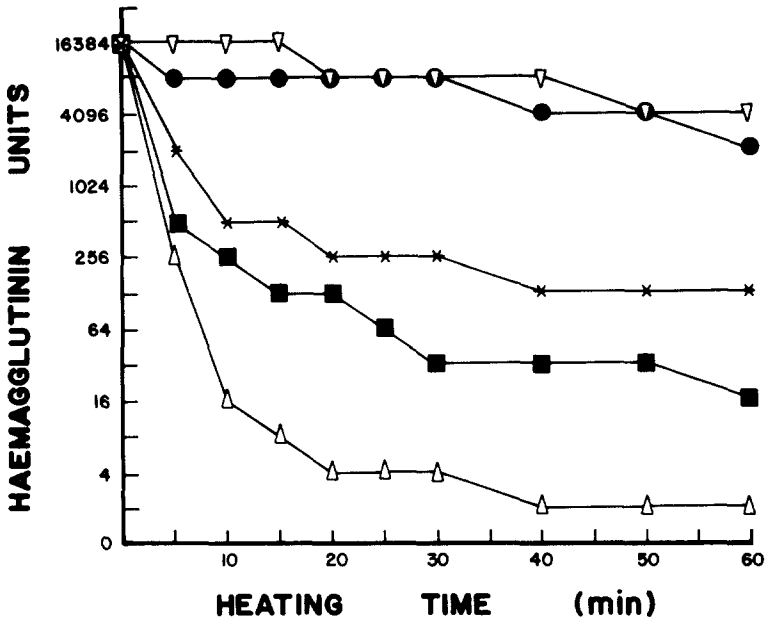


Fig. 2. Inactivation patterns of haemagglutinating activity by raw extracts of lectins from the genetically-improved bean cultivar (FM-RMC). (Key as in Fig. 1).

TABLE 2

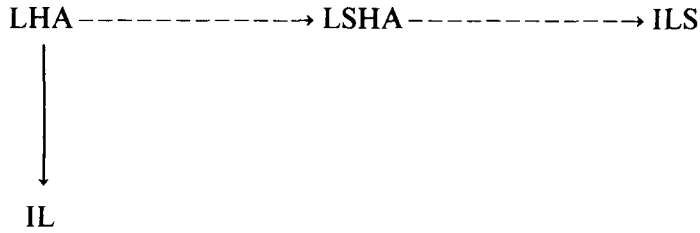
Calculated Values of α_1, α_2 and β Parameters of Eqns (3) and (4) for the Experiments of Figs 1 and 2, Respectively

Temperature tested for HA inactivation[a] (°C)	α_1		α_2		β	
	FM-C[b]	*FM-RMC*[b]	*FM-C*	*FM-RMC*	*FM-C*	*FM-RMC*
65	0·10	0·20	0·009	0·018	0·1000	0·2000
70	0·16	0·23	0·016	0·020	0·0900	0·1900
75	0·26	0·38	0·025	0·027	0·0150	0·0130
80	0·39	0·60	0·030	0·040	0·0040	0·0070
85	0·70	0·72	0·031	0·040	0·0008	0·0050

[a] At the treatment times indicated in 'Materials and Methods'. HA = Haemagglutinating activity.

[b] FM-C = normal bean variety; FM-RMC = genetically-improved bean variety.

following hypothetical scheme can be proposed to account for the biphasic reaction of thermal inactivation of HA:



where:

LHA = amount of integral molecules of lectins with HA in time t.
IL = amount of inactivated lectins.
LSHA = amount of lectin subunits with HA generated from the integral molecules in time t.
ILS = amount of inactivated lectin subunits.

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